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Original Article

THE EFFECT OF VITAMIN D AND FOKI VITAMIN D RECEPTOR POLYMORPHISM TO 25 (OH) D, TNF-A AND NITRIC OXIDE LEVELS IN DIABETIC FOOT PATIENTS

RAFLIS[1], HENDRO SUDJONO YUWONO[2], YANWIRASTI[3] & EVA DECROLI[4]

[1]*Department of Surgery, Faculty of Medicine, Universitas Andalas, Padang, West Sumatera, Indonesia*

[2]*Department of Surgery, School of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia*

[3]*Department of Anatomy, Faculty of Medicine, Universitas Andalas, Padang, West Sumatera, Indonesia*

[4]*Department of Internal Medicine, Faculty of Medicine, Universitas Andalas, Padang, West Sumatera, Indonesia*

ABSTRACT

Vitamin D receptors thought to reduce the inflammatory process activity of diabetic foot ulcers which is influenced by vitamin D receptor polymorphism (VDR). The inflammatory indicators that play a role, including TNF-α and Nitric Oxide (NO), while the VDR gene that mostly studied concerning type 2 DM is FokI variant. This study aimed to analyze the effect of oral vitamin D administration on levels of 25 (OH) D, TNF-α, and NO through VDR Fok-I genetic polymorphism in patients with diabetic foot ulcers.

This experimental study used the pre and post-test control group design method with 36 diabetic foot patients of West Sumatera, Indonesia, who randomly divided into two groups, namely the group given once only100, 000 IU vitamin D3, and the control group was given once only placebo capsules before blood examination. Each respondent was examined for blood for levels of 25 (OH) D, TNF-α, NO, VDR FokI gene polymorphism (PCR analysis and sequencing). Blood tests were taken before treatment (H0) and four weeks later (H28). The results showed that the most VDRFokI gene polymorphisms were heterozygous mutant types followed by wild-type and homozygous mutants. Giving vitamin D increases levels of 25 (OH) D and NO, but does not reduce levels of TNF-α. There is no effect of VDR FokI gene polymorphism on levels of 25 (OH) D, TNF-α, and NO levels.

CONCLUSIONS

Administration of vitamin D is beneficial in diabetic foot patients to increase levels of 25 (OH) D and Nitric oxide (NO) oxide, but not affected by the VDR FokI genetic polymorphism.

KEYWORDS: Vitamin D, Diabetic Foot Ulcer, VDR FokI Genetic Polymorphism & Minangkabau

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INTRODUCTION

The most common complications of diabetes mellitus is the most diabetic feet with a prevalence of 25% (WHO, 2016; Boulton, 2008). Biomolecularly, this condition triggers the secretion of proinflammatory cytokines, especially TNF-α. Endothelial dysfunction will cause a decrease in vascular vasodilation due to a decrease in production and bioactivity of local vasodilation factors, especially nitrogen monoxide (NO) and trigger atherosclerosis (Zhang, 2008; Zhang, 2009; Boulton, 2008).

Vitamin D is very economical in price, so, for this reason, vitamin D3 becomes beneficial and has a high value in public health problems. Most of vitamin D comes from endogenous production in the skin in the form of 7-dehydroxycholesterol, which during exposure to ultraviolet light changes to vitamin D3 (cholecalciferol) and finally metabolites in the form of 24.25-(OH) 2D. A small portion comes from the diet, mainly from fish oil, in the form of vitamins D2 and D3, then metabolized in the liver to form 25 (OH) D, which can be measured in serum to get an overview of vitamin D status (Hewison, 2012). Vitamin D activity in cells caused by the presence of vitamin D receptors (VDR) in various organs including pancreatic beta cells and vascular endothelial cells (Griz et al. 2014). The presence of VDR gene polymorphism affects vitamin D activity in cells. Four types of VDR genetic polymorphisms often found, namely: FokI, BsmI, ApaI, and TaqI. A meta-analysis found that there was a strong relationship between the VDR FokI gene polymorphism and type 2 DM (Yu et al., 2016).

This study aimed to analyze the effect of vitamin D administration and VDR FokI gene polymorphism to 25 (OH) D, TNF-α, and NO levels.

MATERIALS AND METHODS

Ethical Approval

The study protocol has been approved for human study, according to Helsinki Declaration II by the Ethical Committee of the School of Medicine, Universitas Andalas, Padang, West Sumatera, no: 297/KEP/FK/2016.

Study Design

The study was an experimental study with the method of pre-test and post-test control group design.

Participants and Inclusion Criteria

The participants were of West Sumatera province, Indonesia, with foot ulcer of diabetes mellitus type 2.

Inclusion Criteria

The participants were with age 40-60-year-old, diabetes mellitus type 2 with a foot ulcer, outpatient, agree to participate in the study and follow the rules.

Exclusion Criteria

Diabetes mellitus type 2 patients with serious organ diseases, sepsis and bed rest patient.

Sample Number

We used the formula

$$n_1 = n_2 = 2\left[\frac{(z_\alpha + z_\beta)d}{x_1 - x_2}\right]^2$$

Where,

n = sample number

Zα = degree of confidence (α=0,05 Zα=1,96)

Zβ = power (β=80% Zβ= 0,842)

X_1-X_2= clinical judgment (15.3) (Sugden, 2007)

d= deviation standard (14.69) (Sugden, 2007)

The formula resulted in n = 18.

Oral Vitamin D3 Group and the Placebo Group

The grouping of participants were randomly divided into two groups, i.e., Vitamin D3 group and the Placebo group. Vitamin D3 with a single oral dose of 2 capsules (each capsule of D-Vit contains 50,000 IU vitamin D3, GraciaPharmindo, Bandung, Indonesia) were taken once only after breakfast on day-1. The control group took once two placebo capsules after breakfast on day-1. All capsules were given before the blood taken for laboratory examination.

25 (OH) D Measurement

The level of 25(OH)D in the serum was measured by using electroimmunoassay(Diagnostic Biochem, Canada)in COBAS machine in Biomedical Laboratory, School of Medicine, UniversitasAndalas, Padang.

Nitric Oxide Measurement

The blood nitric oxide (NO) level indirectly measured by using NO level measurement kit through the use of spectrophotometry. The kit bought from Stress Marq Biosciences, Cadboro Bay, Victoria, Canada.

TNF-α Measurement

TNF-α serum level was measured using ELISA kit bought from Bioassay Technology Laboratory, Shanghai 200090 China.

The Statistical Data Analysis

The data analyzed applying SPSS version 24.0. The p-value less than 0.05 regarded significant.

The data characteristics, the concentration of vitamin D, VDR FokI gene polymorphism analyzed using chi-square test. The effect of VDR FokI gene polymorphism and TNF-α and NO analyzed using ANOVA test.

Vitamin D Receptor (VDR) FokI

The instrument used was Polymerase Chain Reaction (PCR), and electrophoresis resultedin a standard (FF), homozygous mutant (ff), heterozygous mutant (Ff). The kit for PCR used to buy from PureLink™ Genomic DNA Mini Kit Invitrogen (cataloguenumber K182001).

Informed Consent of the Participant

All subjects will be informed about research procedures, especially parents or guardians who are responsible. If they do not understand it will be given a question and answer time, and if they agree they will sign a letter of agreement.

The laboratory examination performed in the Laboratory of Biomedicine, School of Medicine, UniversitasAndalas, JalanPerintisKemerdekaan no. 94, Padang, West Sumatera, Indonesia.

Vitamin D Receptor (VDR) FokI Amplification

DNA was isolated from 640µl peripheral blood using PureLink™ Genomic DNA Mini kit (Invitrogen). **Amplification of VDR FokI gene was performed using Polymerase Chain Reaction (PCR)** method. The composition

of the PCR solution consisted of 12.5µl Go Tag Green Master Mix (Promega), 1µl Forward VDR FokI Primer forward 5'-CCTGGCACTGACTCTGGCTC-3' (10µM), 1µl Primer Reverse 5-'ACACCTTGCTTCTTCTCCCTC-3' (10µM), 3µl DNA and 7.5µl Nuclease-Free Water, resulting in a total reaction volume to 25µl. One PCR cycle is a series of denaturation, initial denaturation, annealing, elongation, and final elongation processes. The process begins with denaturation at 95^{o}C for 3 minutes, and continues with an initial denaturation at 95^{o}C for 30 seconds. Furthermore, the annealing process was carried out at 59^{o}C for 30 seconds and continued by elongation at 72^{o}C for 55 seconds and ending the final elongation process at 72^{o}C for 5 minutes. This cycle repeated as many as 35 cycles. The size of the PCR product was 265 bp. The electrophoresis process was carried out for 60 minutes at 1.5% agarose using 120V voltage.

RFLP-PCR using Fok1 Restriction Enzyme

In this study, the method used is the Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR). The restriction was carried out using the FokI Restriction Enzyme. The composition of the restriction reaction consisted of 3µl Amplicon PCR, 2µl Green Buffer, 1µl FokI Restriction Enzyme, and 24µl Nuclease-Free Water so that the total volume of restriction reactions was 30µl. Then the electrophoresis process was carried out for 60 minutes on agarose with a concentration of 1.5% with a voltage of 120 V.

Description of VDR FokI Gene Restriction Results

In this study, the VDR gene examined was the FokI gene, where FF showed the standard type (wild-type) with the TT base found in both alleles. Polymorphism can be a homozygous and heterozygous form. Heterozygous form, if there is 1 different allele due to a 1-base change in the gene, that is, base T is replaced by base C so that it becomes TC, with the phenotype Ff. Homozygous form, if there are 2 alleles that experience the same base changes, that is, the second base T is replaced by base C so that it becomes CC on that gene with the phenotype ff.

Gene Sequencing of VDR FokI

Confirm the results of the restriction, sequencing of the PCR results from 3 samples, each representing a group, wild-type, homozygous mutant, and heterozygous mutant, was sequenced. The PCR product examined as a sequence by using a sequencer by the Macrogen Laboratory, in South Korea.

Informed Consent of the Participant

All subjects will be informed about research procedures, especially parents or guardians who are responsible. If they do not understand it will be given a question and answer time, and if they agree they will sign a letter of agreement.

The laboratory examination performed in Laboratory of Biomedicine, School of Medicine, UniversitasAndalas, JalanPerintisKemerdekaan no.94, Padang, West Sumatera, Indonesia.

RESULTS

The participant characteristics in Table 1 as follows.

Table 1: Participant Characteristics

Variable	Intervention f %	Control f %	p
Age (year)			
40-49	6 (33.3)	8 (44.4)	0.781
50-59	8 (44.4)	7 (38.9)	
60	4 (22.2)	3 (16.7)	
Male	9 (50.0)	8 (44.4)	1.00
Female	9 (50.0)	10 (55.6)	
Education			
Elementary	6 (33.3)	7 (38.9)	1.00
High school	12 (66.7)	11 (61.1)	
Job			
Yes	10 (55.6)	11 (61.1)	1.00
No	8 (44.4)	7 (38.9)	
Economy			
Low	7 (38.9)	6 (33.3)	1.00
High	11 (61.1)	12 (66.7)	
Diabetes in family history			
Yes	14 (77.8)	9 (50.0)	0.165
No	4 (22.2)	9 (50.0)	
Treatment frequency			
Regularly	8 (44.4)	10 (56.6)	0.739
Irregularly	10 (55.6)	8 (44.4)	
Wagner classification			
Grade 1	5 (62.5)	3 (37.5)	
Grade 2	4 (36.4)	7 (63.6)	0.336
Grade 3	7 (46.7)	8 (53.3)	
Grade 4	2 (100.0)	0 (0)	
Fasting blood sugar (mg/dL)			
< 100	2 (11.1)	3 (16.7)	0.145
100-125	1 (5.6)	5 27.8)	
≥ 126	15 (83.3)	10 (55.6)	
HbA1c≥ 6.5	18 (100.0)	18 (100.0)	-

DNA Isolation and RFLP-PCR

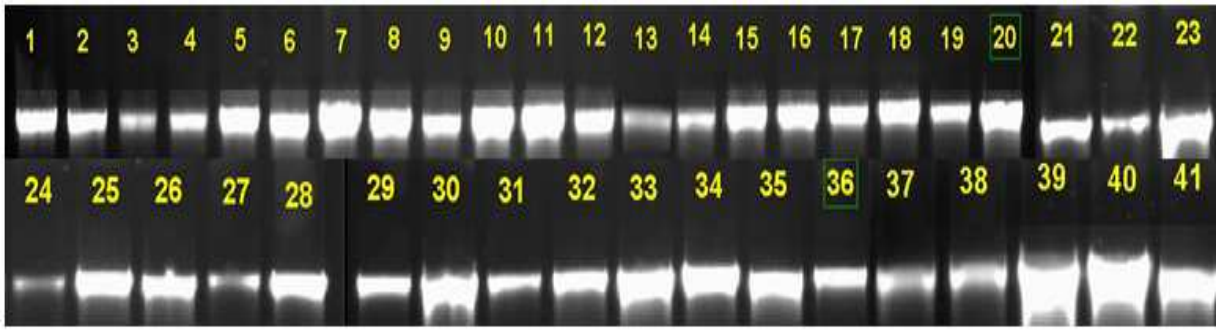

Figure 1: An Electropherogram of DNA Isolation in Patients with Diabetic Foot Ulcer

DNA isolation was carried out using the PureLink ™ Genomic DNA Mini Kit resulted in good quality DNA as shown in the Figure 1. By using VDR FokI forward and reverse primers as mention in the Materials and Methods, PCR product was produced with the size of 265 bp as it was shown in Figure 2.

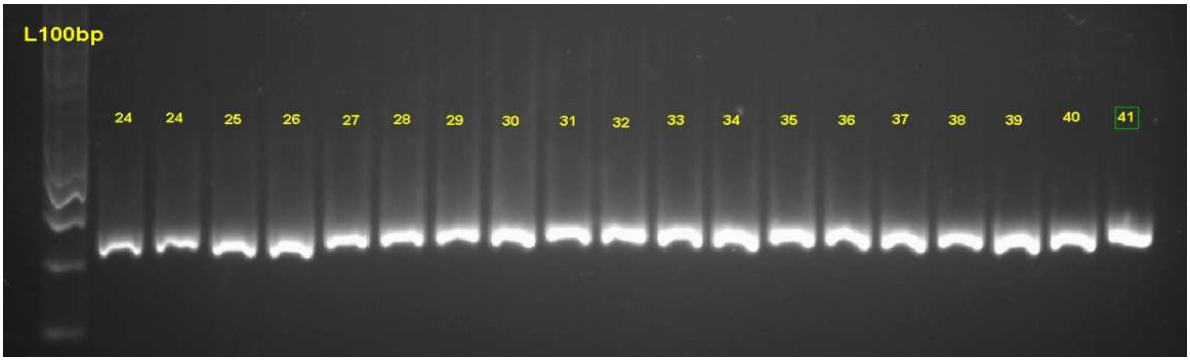

Figure 2: Electropherogram of VDR Gene with PCR Amplification Length 265 bp

The Picture Shows the DNA Amplification Band (PCR) at 265 bp, Which Seen between the Ladder 200 bp - 300 bp

Restriction Fragment Length Polymorphism-PCR (RFLP-PCR)

The Figure 3 was the result of electrophoregram restriction of the VDR FokI gene of sample no.1-41.

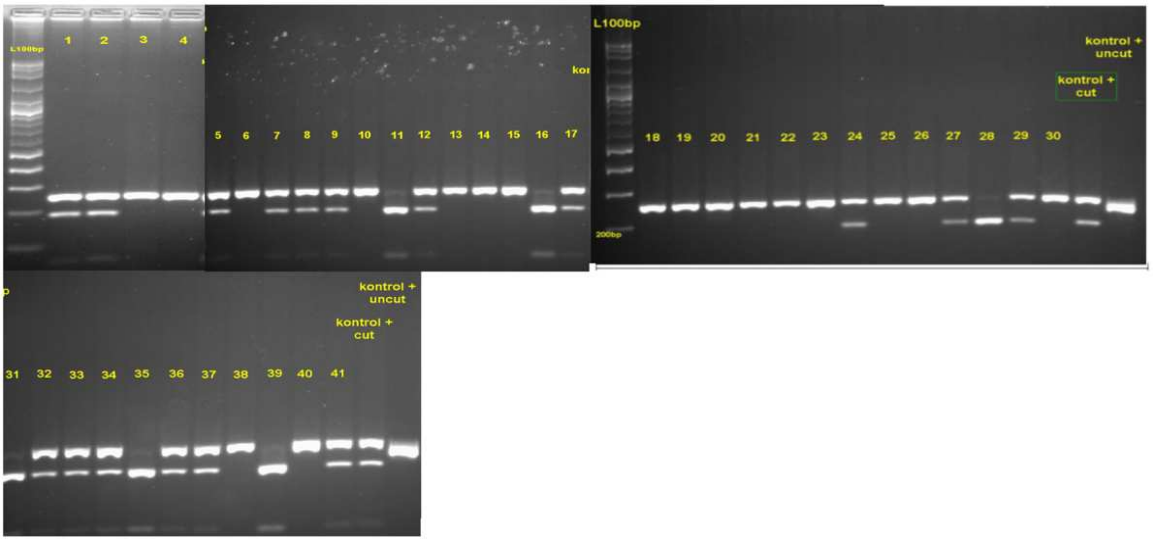

Figure 3: Electrophoregram Restriction (RFLP-PCR) of the VDR FokI Gene of Sample No. 1-41

Sanger Sequencing Results

To confirm the RFLP-PCR results, one of each samples with the genotype wild type (T/T), heterozygous (T/C) and homozygous mutant (C/C) were Sanger Sequenced using forward and reverse primers. The Sanger sequencing results for each genotype mention above were shown in the Figure 4.

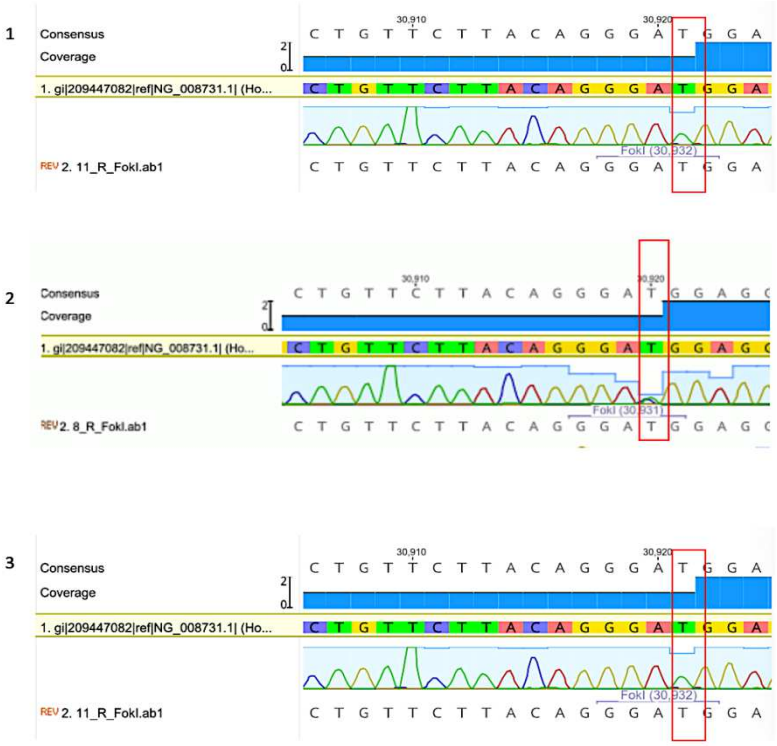

Figure 4: No.1- VDR FokI Gene Wild-Type (T/T);
No.2- VDR FokI gene Heterozygous Mutant: (T/C);
No.3- VDR FokI gene Homozygous Mutant (C/C)

Genotyping of VDR Fok1 Gene Polymorphism

The frequencies of wild-type and heterozygous mutant VDR FokI genes genotype were found to be almost the same in the treatment and control groups. However, the frequency of homozygous mutant getypes were the lowestof the treatment group (40% and 60%), but not statistically significant (p = 0.875).

Table 2: Spreading of the VDR FokI Gene Polymorphism

	Intervention n	Control n	p
Wild-type	8(53.3%)	7(46.7%)	0.875
Heterozygousmutant	8(50.0%)	8(50.0%)	
Homozygousmutant	2(40.0%)	3(60.0%)	

The Effect of VDR Fok1 Gene Polymorphism to the Levels of NO, TNF-a and 25(OH)D

Statistically, there was no effect of VDR FokI gene polymorphism on NO, 25(OH)D, TNF-αlevels after vitamin D3 administration (p> 0.05).

The wild-type and heterozygous mutant VDR FokI genes were found to be almost the same in the treatment and control groups. However, homozygous mutant types were the lowest in the treatment group (40% and 60%), but not statistically significant (p = 0.875).

The Effect of VDR Fok1 Gene Polymorphism to the Levels of NO, TNF-a and 25(OH)D

Statistically, there was no effect of VDR gene polymorphism on NO, 25(OH)D, TNF-α levels after vitamin D3 administration (p> 0.05).

Table 3: Mean Difference of NO, 25(OH)D, TNF-α Levels after Vitamin D3 Administration Based on VDR FokI Gene Polymorphism

VDR FokI Polymorphism		Mean Difference Intervention		Mean Difference Control	
		Level± SD	p-Value	Level± SD	p-Value
NO ng/dl	Wild-type (FF)	8.80±42.18	0.903	-40.42 ± 43.31	0.171
	Homozygous mutant (ff)	-8.71±10.73		-97.84 ± 106.47	
	Heterozygous mutant (Ff)	5.40±57.79		-25.24 ± 37.27	
25(OH)D ng/dl	Wild-type (FF)	3.80 ± 7.42	0.914	1.24 ± 2.77	0.218
	Homozygous mutant (ff)	3.00 ± 5.87		-2.7 ± 5.04	
	Heterozygous mutant (Ff)	4.50 ± 7.53		-0.15 ± 3.49	
TNF-α ng/dl	Wild-type (FF)	4,87 ± 35,46	0.63	3.06 ±45.83	0.987
	Homozygous mutant (ff)	40,29 ± 68,93		0,43 ± 35,65	
	Heterozygous mutant (Ff)	7,55 ± 52,29		0,24 ± 21,19	

Statistically, there was no effect of VDR gene polymorphism on the serum levels of NO, 25(OH)D, TNF-αafter vitamin D3 administration (p> 0.05).

Table 4: Effect of Vitamin D Oral Administration on NO, 25(OH)D, TNF-α Levels

	Intervention		Control		p
	Before	After	Before	After	
NO (ng/dL)	86.07±37.14	91.41±41.65	116.95 ± 56.99	73.71 ±26.79	0.008 (significant)
	ΔMean 5.3453 ±46.30		ΔMean -43.25 ±57.01		
25(OH)D (ng/dL)	27.30 ± 10.62	36.23 ± 10.88	32.52 ± 9.54	31.61 ±9.58	0.000 (significant)
	Δ Mean 8.925±6.32		ΔMean - 0.91 ±3.62		
TNF-α (ng/dL)	190.29 ±183.22	200.29 ± 200.31	127.63 ± 27.63	123.31 ± 21.45	0.245
	ΔMean 10.01 ± 45.24		ΔMean -4.32 ± 22.92		

Statistically, there is a significant effect of oral administration vitamin D to NO and 25 (OH) D levels in diabetic foot sufferers (p <0.05).

DISCUSSIONS

Demographic Characteristics

In this study, most (44.4%) of diabetic foot sufferers had ages 30-60 years, more or less the same as other studies regarding patients with diabetes mellitus in Padang, Denpasar (Bali Island) and in Manado (Decroli, 2008; Dwikayana, 2016). Overseas diabetic foot ulcer patients were on average 58-year-old (in India, Saseedharan, 2018), in 48-57-year-old (Mariam, 2017).

Polymorphism VDR FokI

The VDR FokI gene is one of the VDR genes that is widely associated with type 2 Diabetes disease and has the potential to experience gene polymorphism with various types of mutants. In this study, there were almost the same types of wild-type and heterozygous mutant VDR polymorphisms in the treatment and control groups. Though, the frequency of homozygous mutant genotype were lower in the treatment group (40% vs.60%), however it was not statistically significant (p = 0.875).

The Chilean study examining the VDR FokI gene polymorphism in patients with type 2 and non-Diabetes patients, found that there was a significant difference in the frequency of the mutant allele (f) of the VDR FokI gene polymorphism in type 2 Diabetes groups compared to non-Diabetes groups. This mutant allele has been reported could

increase the risk of suffering from type 2 diabetes 1.9 times (Angel B, 2018). The study of type 2 Diabetes patients in Iran, got almost the same results, namely the distribution of VDR FokI polymorphisms were FF (41.5%), followed by Ff (33.5%) and ff (25%) (Neyestani TR, 2013). Consistent findings also found in studies with diabetic foot sufferers, where TT (wild-type) and TC (heterozygous Ff mutants) genotypes were 1.76 times higher than CC (homozygous ff mutants) in diabetic foot patients compared to footless DM diabetic (Soroush N, 2016). Studies in India in type 2 and non-Diabetes patients, found that 60% of Diabetic patients had Ff heterozygous mutant polymorphisms, but were low in homozygous ff mutants (2%) (Bid HK, 2009).

The VDR gene is a nuclear receptor located on chromosome 12q13 (Uitterlinden et al., 2004). Single nucleotide polymorphisms from the VDR FokI (rs 2228570; C/T) are located in exon 2, containing T allele changes to C. Polymorphism located in the initial codon (ATG), and there is a protein C variant, so changes in these alleles will form proteins of different sizes (Valdivielso, 2006). The VDR FokI gene polymorphism is the only VDR polymorphism that is different from other VDR genes which at the time of translation formed two protein products and the only form of VDR gene polymorphism that is not related to other VDR gene variants, so this makes it unique (Neyestani et al., 2013).

The FokI polymorphism is significantly associated with diabetes complications. The T allele in FokI is seen to be associated with an increased risk of diabetic retinopathy and a biomarker that can predict the risk of diabetic retinopathy in type 2 Diabetes patients in the Han ethnic population in China (Zhong et al., 2015). A meta-analysis study shows that FokI polymorphisms play a role in susceptibility to diabetic nephropathy in the Caucasian population (Liu et al., 2014). It was also observed that patients with diabetes and diabetic foot had a high prevalence of severe vitamin D deficiency than those with diabetic foot diabetes (Zubair et al., 2013; Tiwari et al., 2013).

The FokI VDR gene polymorphism found in allele differences has a genetic contribution to type 2 Diabetes due to its effect on insulin secretion. These results show that the subjects of this study who were diabetic foot ulcer sufferers had T to C mutations in the FokI gene, which indirectly became a risk factor for the occurrence of type 2 Diabetes and diabetic foot ulcer (Palomer, 2008; Angel B, 2018).

Vitamin D has important effects on insulin action. Vitamin D has an influence on the occurrence of type 2 diabetes mellitus through its influence on insulin metabolism. In addition, vitamin D has a strong influence on the safety of many metabolic pathways in the formation of type 2 diabetes mellitus. This study resulted in the influence of Vitamin D supplementation affecting NO levels, which can mean good because NO is a vasodilator that can improve among them is the vasodilation of penile blood vessels, and inflammatory prevention (in this study did not affect TNF-α) (Talib 2017; Khazan 2015). In diabetics the anti-inflammatory effects of NO are very beneficial (Talib 2017). The high level of NO is toxic for health and can cause bodily dysfunction (Khazan 2015). Fortunately, the NO levels produced by a supplement vitamin D will not produce excessive NO levels.

CONCLUSIONS

- VDR FokI gene polymorphism found in the diabetic foot study samples were mostly wild-type and heterozygous mutant followed by homozygous mutant types.
- VDR FokI gene polymorphism does not affect the level of Nitric Oxide, 25 (OH), and DTNF-α after administration of vitamin D in diabetic foot sufferers.

- Vitamin D can increase levels of 25 (OH) D, and Nitric Oxide in diabetic foot sufferers, but not affect TNF-α.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

REFERENCES

1. *Angel B, Lera L, Márquez C & Albala C 2018. The Association of VDR Polymorphisms and Type 2 Diabetes in Older People Living in Community in Santiago de Chile. Nutrition and Diabetes;8(31):1-9*
2. *Bid HK, Konwar R, Aggarwal CG etal, 2009. Vitamin D Receptor (FokI, BsmIadnTaqI) Gene Polymorphisms and Type 2 Diabetes Mellitus: A North Indian Study. Indian Journal Medical Science;63(5):187-94*
3. *Boulton, Andrew J. M. 2008. The Diabetic Foot: Grand Overview, Epidemiology, And Pathogenesis. Diabetes/Metabolism Research And Reviews;24(Suppl 1):S3-6*
4. *Decroli E, Karimi J, Manas S, Syahbuddin S, 2008. Profil Ulkus Diabetik pada Penderita Rawat Inap di Bagian Penyakit Dalam RSUP Dr. M. Djamil Padang. Majalah Kedokteran Indonesia;58(1)*
5. *Dwikayana M, Subawa AAN, Yasa WPS, 2016. Gambaran HbA1c Pasien Diabetes Mellitus Tipe 2 Dengan Komplikasi Ulkus Kaki DiabetikDiPoliklinikPenyakit Dalam RSUP Sanglah Denpasar Periode April-September 2014. E-Jurnal Medika;5(7)*
6. *Griz, L. H.M., Bandeira, F., Gabbay, M.A.L., Dib, S.A. and Carvalho, E.F.D., 2014. Vitamin D and Diabetes Mellitus: An Update 2013. Arquivos Brasileiros De Endocrinologia & Metabologia, 58(1): 1-8.*
7. *Hewison M. An update on vitamin D and human immunity. Clinical Endocrinology. 2012,76(3):315-25*
8. *Li L, Wu B, Liu JY, Yang LB. Vitamin D receptor gene polymorphisms and type 2 diabetes: a meta-analysis. Arch Med Res. 2013; 44: 235-41.*
9. *Mariam TG, Alemayehu A, Tesfaye E, Mequannt W, Temesgen K, Yetwale F, et al. 2017, Prevalence Of Diabetic Foot Ulcer And Associated Factors Among Adult Diabetic Who Attend The Diabetic Follow-Up Clinic At The University Of Gondar Referral Hospital, North West Ethiopia, 2016 : Institutional-Based Cross-Sectional Study. Hindawi, Journal of Diabetic Research, ID 2879249, 2017:1-8*
10. *Al-Khafaji, J. K. T. In Vitro Inhibitory Efficacy of Cranberry Fruit on Escherichia Coli and Proteus Mirabilis Isolated from UTI Patients in Hilla City.*
11. *Neyestani TR, Djazavery A, Eahraghian MR et. al, 2013. Vitamin D Receptor FokI Polymorphism Modlates Diabetic Host Response to Vitamin D Intake. Diabetes Care; 36:550-6*
12. *Palomer, X, Et Al. 2006. Role of Vitamin D In The Pathogenesis Of Type 2 Diabetes Mellitus. Diabetic, Obesity, And Metabolism; 10:185-197*
13. *Saseedharan S, Sahu M, Chaddha R, Pathrose E, Bal A, Bhalekar P, et al. 2018. Epidemiology of diabetic foot infections in a reference tertiary hospital in India. Brazilian Journal of Microbiology. 49(2018):401-6*
14. *Soroush N, Radfar M, Hamidi AK, Abdollahi M, Qorbani M, Razi F, et al., 2017. Vitamin D Receptore Gene FokI Variant in Diabetc Foot Ulcer and its Relation with Oxidative Stress. Gene; 599:87-91*
15. *Tiwari S, Pratyush DD, Gupta SK, Singh SK. Vitamin D Defficiency is Associated with Inflammatory Cytokine Concentrations in Patients with Diabetic Foot Infection. British Journal of Nutrition. 2014; 112(12): 1938-43*

16. *Uitterlinden AG, Fang Y, Meurs J etal, 2004. Vitamin D ReceptorGenePolymorphism in Relationto Vitamin D RelatedDisease States. Journalof Steroid Biochemistry&MolecularBiology; 89-90: 187-93*
17. *Aziz, I. M. A. Brucella Infections in Patients Hospitalized for Fever: A Serological Study at Al Delengat Fever Hospital, Damanhur, Egypt.*
18. *Valdivielso JM, Fernandez E, 2006. Vitamin D ReceptorPolymorphismandDisease. ClinicaChimica Acta; 371:1-12*
19. *WHO.2016. Global Report on Diabetes. Http://Www.Who.Int*
20. *Yu, Fei, Cui LL, Li X, Wang CJ, Ba Y, Wang L.et al. 2016. The Genetic Polymorphism in Vitamin D Receptor And The Risk Of Type 2 Diabetes Mellitus: An Updated Meta-Analysis. Asia Pac.J.Clin.Nutr; 25(3): 614-624*
21. *Zhang, C.2008. The Role Of Inflammatory Cytokines In Endothelial Dysfunction. Basic Res Cardiol;103(5): 398-406*
22. *Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J., et al. 2009.Role Of TNF- α In Vascular Dysfunction. Clinical Science; 116:219-30*
23. *Zhong X, Du Y, Lei Y,Liu N, Guo Y, Pan T. 2015. Effectof Vitamin D Recepto rGene Polymorphism and Clinical Characteristicson Risk of Diabetic Retinopathy in Han Chinese type 2 Diabetes Patients. Gene.566(2):212-6*
24. *X, R. A. (2014). Personality pattern of female cardiovascular patients and cancer Patients: an analytical study. Natural and Social Sciences, 2, 61-72.*
25. *Zubair M, Malik A, Meerza D & Ahmad J, 2013. 25-Hydroxyvitamin D (25(OH)D) LevelsandDiabeticFootUlcer: Is There Any Relationship? Diabetes &MetabolicSyndrome : ClinicalResearch&Reviews; 7:148-153*
26. *Talib RA, Khalafalla K., Canguven O. The role of vitamin D supplementation on erectile function. Turk J Urol 2017; 43(2): 105-11*
27. *Khazan M, Hdayati M. The role of nitric oxide in health and diseases. Scimetr.2015.3(1):e20987*